

Settling of Crawlers of *Bemisia tabaci* (Homoptera: Aleyrodidae) on Five Vegetable Hosts

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ABSTRACT The nymphal stage of whitefly pests is important in terms of its relationship to pest management measures and virus epidemiology. Among the nymphal forms, the active first instar, i.e., crawler, is the only mobile form of immature whiteflies. A study was conducted to determine any influence of vegetable plant species and temperature on net distance moved (between the egg site and final resting site) by crawlers of the B-biotype sweetpotato whitefly, *Bemisia tabaci* (Gennadius). Tests were conducted in the greenhouse and under controlled laboratory conditions on five vegetable hosts: cantaloupe, *Cucumis melo* L.; collard, *Brassica oleracea* ssp. *acephala* de Condolle; cowpea, *Vigna unguiculata* (L.) Walpers; pepper, *Capsicum annuum* L.; and tomato, *Lycopersicon esculentum* Miller. The net distance moved was shortest on collard in which the crawler ceased traveling ≈ 2 mm from where it hatched. The crawler ceased traveling ≈ 10 – 15 mm from the site of hatching on the other vegetable host plants. Observations on collard in the laboratory indicate that the crawlers traveled 21 min during the first stadium. No effect of constant temperature over the temperature range of 16–34°C was detected on the net travel distance of the crawlers. These data suggest that among the plant species in this study, collard is highly attractive for feeding and/or it offers suitable feeding sites that are easy to locate by the crawler. The results of this study help define the behavior of crawlers on several host plants.

KEY WORDS *Bemisia argentifolii*, *Bemisia tabaci*, behavior, crawler, whitefly, vegetable

WHITEFLIES ARE DESTRUCTIVE pests on a global scale on numerous row, horticultural, and other crops. The whitefly problem has been particularly troublesome over the past decade because of infestations by the B-biotype of the sweetpotato whitefly, *Bemisia tabaci* (Gennadius) (USDA 1997). This whitefly is synonymous with *B. argentifolii* Bellows and Perring (Perring et al. 1993, Bellows et al. 1994, Brown et al. 1995). The B-biotype *B. tabaci* was first observed in Florida in the late 1980s (Schuster et al. 1990). This insect has a wide diversity of hosts (Greathead 1986, Cock 1993, Simmons et al. 2000), but the acceptance and performance of *B. tabaci* varies among hosts. The crawler, i.e., the active first instar of Aleyrodidae before it settles (Borror et al. 1981), is the only mobile immature form of whiteflies. Price and Taborsky (1992) reported on the distance that *B. tabaci* crawlers traveled on the host leaf of poinsettia, *Euphorbia pulcherrima* Willd. ex Klotzsch, in the greenhouse. In their study, crawlers of *B. tabaci* traveled a short distance (≈ 1 mm) after eclosion before settling. Moreover, on poinsettia leaves, over 50% of the crawlers settled within 3 h of

hatching (Price and Taborsky 1992). In other work, Summers et al. (1996) demonstrated that in the absence of food (i.e., on a senescent leaf or on a stem of the plant), newly emerged *B. tabaci* crawlers readily traveled to and settled on leaves over 50 mm from source of origin.

Not only can lack of food affect crawler movement, but light can also be a factor. Summers (1997) demonstrated that crawlers of *B. tabaci* exhibit positive photo tactic behavior. Eggs of *B. tabaci* are deposited in various proportions on both leaf surfaces of many hosts, but are generally on the lower leaf surface (Lynch and Simmons 1993, Simmons 1994). The percentage of *B. tabaci* crawlers which moved between leaf surfaces varied among crawlers on five species of vegetable hosts (Simmons 1999), but no data were collected on the distance traveled on these vegetable leaves.

Price and Taborsky (1992) noted several reasons (including contact with biological and chemical control agents, and vectoring) why crawler movement are important in whitefly management and epidemiology. In addition, knowledge of crawler settling behavior may be useful in host plant resistance studies. If a food source is available and highly acceptable, the crawler may travel only a short distance before settling, but on a host which is less favorable for feeding or if there is

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difficulty finding a feeding site, there might be an increase in travel by crawlers. The objectives of this study were to determine any influence of host species on the net distance traveled by crawlers of B-biotype *B. tabaci* on leaves of certain vegetables, to determine any influence of temperature on its net travel distance, and to determine the duration of travel. The host plant species used in this study were selected based on their diversity in terms of whitefly acceptance and performance, and plant morphology.

Materials and Methods

Plant Materials and Source of Insects. The five vegetable host plants used in the experiment were cantaloupe, *Cucumis melo* L., 'Top Mark'; collard, *Brassica oleracea* ssp. *acephala* de Condolle, 'Georgian'; cowpea, *Vigna unguiculata* (L.) Walpers, 'Mississippi Silver'; pepper, *Capsicum annuum* L., 'Keystone'; and tomato, *Lycopersicon esculentum* Miller, 'Homestead.' These are all hosts for *B. tabaci* and are hosts on which whitefly behavior and performance varies (Simmons 1994, 1999). The plants were established in black plastic pots (7.5 cm by 7.5 cm wide by 5.5 cm deep) filled with Jiffy Mix (Hummert's, St. Louis, MO). All plants were watered daily and were fertilized once with liquid fertilizer (Peter's Excel, Hummert's). The plants were grown in a whitefly-free greenhouse for the duration of the experiment except as noted below.

Whiteflies used in the study were from a colony that originated in Charleston, SC from a feral population on sweetpotato, *Ipomea batatas* (L.), in 1992. Thereafter, the insects were continuously reared on uncaged assorted vegetables (Simmons 1994) in a greenhouse. Each fall, supplemental B-biotype *B. tabaci* from sweetpotato were added to the colony to increase genetic diversity.

Crawler Net Travel Distance. Net travel distance is defined herein as the distance between the site of the egg and the final resting site of the nymph. However, unless a crawler only travels in a straight line, the test for net travel distance cannot determine the total distance traveled by a crawler. The site of the egg was based on the presence of the empty eggshell, which was still attached to the leaf in each instance during the study. Upon reaching the first trifoliate stage in cowpea, the second true leaf stage for cantaloupe, and the third true leaf stage of collard, tomato and pepper, the plants were moved to the laboratory for infestation with whiteflies. The youngest leaf of each plant species was caged in a modified petri dish, except only the terminal leaflet for tomato and the middle leaflet of the trifoliate leaf of cowpea were caged. The leaves or leaflets remained attached to the plant during the test. Each cage consisted of a petri dish (15.2 cm diameter by 2.2 cm deep) with an opening drilled in the lid to facilitate insertion of whiteflies. A U-shaped opening was made in the side of the dish to allow the petiole of the leaf a place to rest while the leaf or leaflet was inside of the dish. This opening was lined with cotton to help protect the stem from injury and to prevent insect escape. The stems of all test leaves were tagged.

Ten adult female *B. tabaci* from the greenhouse colony were aspirated into each cage and the entrance was plugged with cotton. The insects were held 1–2 h in the cages. The leaf was then removed from the cage and all adult whiteflies were aspirated from the leaf. Using forceps, all but one egg was removed from the lower leaf surface and all eggs were removed from the upper leaf surface of each test leaf. An attempt was made to leave a test egg that was away from the margin of leaves. The plants were held in the laboratory for 2–3 d during the egg removal process and were returned to and held in the greenhouse until 11 d after oviposition. The egg of the B-biotype *B. tabaci* hatches in 7.1 d at 24°C on cotton (Wagner 1995). The plants were watered as needed each day without wetting the leaves. The temperature in the greenhouse ranged from 25 to 35°C. No supplemental light was provided. The duration of daylight, from sunrise to sunset, ranged from 12–14 h during the test.

The distance from the outer margin of the sessile nymph to the eggshell was measured with a digital caliper under a microscope. Data were recorded on whether the eggshell was on the apical or basal half of the leaf, and whether the eggshell was on the margin of the leaf (≈ 10 mm from edge of leaf) or not. To aid in the relocation of the eggshell, two reference points about 20 mm apart were marked with a red colored fine point Sharpie permanent marker (Series No. 30000; Stanford, Bellwood, IL). The reference points were made on opposite sides of the egg so that the egg was between the two marks. After the distance was measured, the leaf area of each leaf was measured using a leaf-area meter (model 3000, LI-COR, Lincoln, NE). All plant species were tested simultaneously and 10 plants of each species were tested per trial. The experiment was repeated six times for a total of 60 eggs per plant species.

All data analyses in this study were conducted using SAS (SAS Institute 1999). Comparisons were made among plant species for mean net travel distance, and between sections of leaf for net travel distance, according to the Student-Newman-Kuels test. Correlation analyses were tested between net travel distance and leaf area.

Duration of Crawling. A test was set up on a collard plant, as described above, for observations on duration of crawling. After 6 d, the plant was moved from the greenhouse to a bench in the laboratory ($24 \pm 2^\circ\text{C}$). The fluorescent ceiling light remained on continuously in the laboratory for this test. An RGB remote-head video camera (LX-450A, Optronics Engineering, Goleta, CA), with a macro lens and attached to a time-lapse recorder and monitor, was focused on the egg on the underside of the leaf. The leaf was recorded by video until the nymph reached the second instar. The video was reviewed and the duration and frequency of movement were recorded. Data were also collected on the net travel distance of the crawlers as described in the above test. The test was repeated 10 times. Because of the relatively long distance traveled by the crawlers on the other hosts in this study, travel duration was not tested on those species.

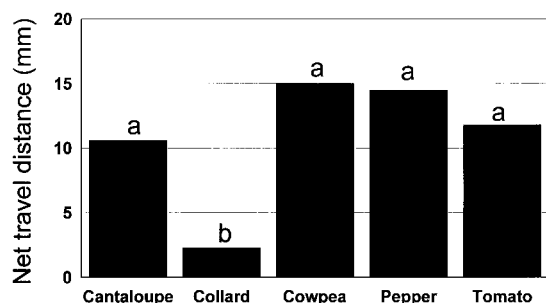


Fig. 1. Mean net travel distance from the ovipositional site to the final resting site by crawlers of B-biotype *B. tabaci* on the lower leaf surface of five vegetable crops. Data bars with different letters are significantly different according to Student-Newman-Kuels' test; $P < 0.001$; $df = 4, 269$; $F = 19.95$.

Test for Temperature Effect on Net Crawling Distance. A test was conducted to determine any influence of temperature on net crawling distance from the oviposition site. Based on crawling data from the above-mentioned tests, seedlings of collard and cowpea were established in the greenhouse as described above. Using the above described infestation method, one egg was left on the lower leaf surface while all other eggs were removed. The plants were held in the laboratory ($24 \pm 1^\circ\text{C}$) for 5–6 d after oviposition to allow initial similar development of all eggs. The plants were placed in each of four environmental chambers (Percival, Boone, IA) under temperature settings of 16, 22, 28, or 34°C . Relative humidity was 75–90%, and lighting (15:9 [L:D] h) was by 40-W cool white fluorescent lamps. The lamps were placed vertically and were located along the side of the chamber, but the bottom 25 cm of the lamps were shaded by cardboard so that the light source which reached the leaf was from above. The plants were situated on a rack at the bottom of the chamber. They were provided water as needed, and were held in the chambers until 10–15 d (depending on temperature) after oviposition. Subsequently, for each leaf, the distance the nymph traveled from the eggshell was measured as noted in the above test on net travel distance.

For each crop, 10 plants were tested per temperature. The test was repeated four times for each temperature. Mean net travel distance was compared among temperatures for each crop using the Student-Newman-Kuels test.

Results and Discussion

Crawler Net Travel Distance. In the greenhouse test, 4.3% of the eggs did not hatch and 0.7% died while emerging from the eggs. Crawlers moved on leaves of all host plant species tested. The shortest ($P < 0.05$) average net travel distance (from site of the egg to the settled position) was observed on collard (Fig. 1). The distance that crawlers traveled on collard averaged 2.4 mm, which was from 4–6 times less than the net distance they traveled on the other four plant species. On the other host plants, the average net travel dis-

Table 1. Net travel distance by crawlers of B-biotype *B. tabaci* between leaf sections on leaves of five plant species in the greenhouse and laboratory

Host	Mean distance crawler settled from egg, mm			
	Hatched on		Hatched on	
	Inner leaf	Near margin	Apical leaf half	Basal leaf half
Greenhouse test				
Cantaloupe	10.18a	10.44a	12.45a	9.46a
Collard	2.61a	2.20a	2.34a	2.44a
Cowpea	14.89a	11.07a	16.40a	14.38a
Pepper	13.73a	17.07a	19.41a	12.61b
Tomato	12.85a	10.10a	12.17a	12.00a
Laboratory test ^a				
Collard	2.18a	2.63a	2.82a	1.77b
Cowpea	7.56a	10.18a	8.46a	7.70a

Means in a row and between leaf sections (inner leaf versus near margin, and apical leaf half versus basal leaf half) and followed by the same letter are not significantly different ($P > 0.05$) according to Student-Newman-Kuel's test (SAS Institute 1999).

^a Laboratory data are combined across four temperatures (16, 22, 28, and 34°C).

tance ranked in increasing order on: cantaloupe, tomato, pepper, and cowpea; the distance traveled on these hosts was not significantly different (Fig 1). Similarly, the range in net travel distance paralleled the rank order of the mean travel distance. The minimum and maximum on the different species were: 0.35–12.05 mm on collard, 1.11–32.32 mm on cantaloupe, 1.29–47.48 mm on tomato, 1.93–50.20 mm on pepper, and 1.27–61.90 mm on cowpea. Within plant species, only on tomato was the net distance traveled correlated ($P < 0.05$; $r = 0.27$) with the size of the leaves. However, the degree of leaf expansion after the crawler traveled was not determined for either plant species.

For each plant species, crawlers that hatched from eggs deposited away from the margin of the leaf settled the same distance as those that originated near the margin of the leaf (Table 1). The crawlers traveled over veins and midribs of leaves of each plant species. Crawlers that originated on the basal and apical halves of leaves traveled the same distance, except for pepper in the greenhouse test and for collard in the laboratory test (Table 1). Some crawlers were observed to remain in the same section of the leaf as the egg, while others moved to a different section. *B. tabaci* can develop to the adult stage on all plant species used in this study, but pepper and cowpea are marginal hosts for its acceptance and development (Simmons 1994, 1999). Cantaloupe is a host that is highly acceptable for adult feeding and nymphal development, and has abundant vascular bundles for feeding sites (Chu et al. 1995, Cohen et al. 1996a, Cohen et al. 1998). In a study with cantaloupe, all instars of *B. tabaci* were found attached to minor vascular bundles (Cohen et al. 1996a).

The net distance traveled by the crawlers on collard is similar to that reported on poinsettia (0.73 mm) by Price and Taborsky (1992). Both of these crops are known to be highly acceptable for adult feeding and

Table 2. Incidence of travel by crawlers of B-biotype *B. tabaci* on collards in the laboratory with continuous overhead fluorescent light ($24 \pm 2^\circ\text{C}$)

Observation	Response	
	Mean	Range
No. of times traveled	2.7 ± 0.5	1–5
Distance between egg and settled nymph	3.26 ± 0.72 mm	0.80–6.42 mm
Duration between travel episodes	2.04 ± 0.70 h	0.08–12.1 h
Duration per travel episode	7.8 ± 1.18 min	1.4–21.5 min
Duration of all travel episodes	21.4 ± 3.11 min	10.0–31.7 min

$n = 10$; values for means are \pm SEM.

nymphal development for *B. tabaci*, and it is apparently easy for the crawlers to find suitable feeding sites on these hosts. Data in a study by Simmons (1999) suggest that collard may offer abundant feeding sites compared with the other hosts in this study. Although those data (Simmons 1999) best describe the response to the crawlers among upper leaf surfaces of the different plant species, the data parallel what was observed herein. Price and Taborsky (1992) observed that on poinsettia, once the crawler became sessile, no additional movement occurred except for a distance of about a body width immediately before pupation. In my study, some of the nymphs settled adjacent to the eggs from which they emerged. Although, the total distance traveled by the crawlers was not ascertained on either plant species, the net travel distance may be positively correlated with the total distance traveled. Price and Taborsky (1992) reported that based on one-hour interval observations in the laboratory, the total distance traveled (1.57 mm) by the crawlers on poinsettia was about twice the net distance traveled in the greenhouse. In laboratory observations on cotton, Cohen et al. (1996b) reported that the crawler did a lot of back-tracking and walked an average of 2,300 $\mu\text{m}/\text{minute}$. They concluded that the crawler is capable of covering the length and width of cotton leaf in about 2 h.

Duration of Crawling. On collard, the crawlers traveled an average of 21.0 min in a laboratory test (Table 2). Among the individuals that were monitored, the net travel distance (3.3 mm) was similar to that observed in the greenhouse test. The crawlers traveled from one to five times with an average duration of 2.04 h between travels, i.e., there were up to five times of stopping to rest, sample the leaf surface, probe, and/or feed (Table 2). Overall, the crawlers traveled 2.7 times during the first stadium.

Test for Temperature Effect on Net Crawling Distance. Ten percent of the eggs in the laboratory temperature test did not hatch, and an additional 1.5% of the crawlers died while emerging from the egg. No significant influence of temperature was detected for net distance of crawler travel (Table 3). In the temperature test on collard, the average net travel distance of crawlers among the temperature treatments ranged from 1.7 to 2.6 mm (Table 3). Hence, the mean net movement of the crawlers on collard was similar to that observed in the greenhouse test. Likewise, the

Table 3. Net distance which crawlers of B-biotype *B. tabaci* settled on two hosts under four constant temperature regimes in the laboratory (mean \pm SEM)

Host	Mean distance crawler settled from egg, mm			
	16°C	22°C	28°C	34°C
Collard	2.56 ± 0.41	2.41 ± 0.40	2.44 ± 0.39	1.69 ± 0.39
Cowpea	8.12 ± 1.20	9.21 ± 1.21	6.03 ± 1.28	8.27 ± 1.14

There was no significant ($P > 0.05$) effect of temperature within either host plant.

range of the net distance moved on collard by individual crawlers in the laboratory temperature test (0.19–16.43 mm) was similar to that observed in the greenhouse (0.35–12.05 mm). Conversely, overall net movement on cowpea in the laboratory tended to be less than what was observed in the greenhouse test. On cowpea, the net distances traveled at the four laboratory temperatures were 6.0–9.2 mm (Table 3). Moreover, the range of the net distance moved by individual crawlers on cowpea in the laboratory (0.75–33.67 mm) was less than what was observed in the greenhouse test (1.27–61.90 mm). Because temperature, within the scope of what was tested, was not found to be a limiting factor, the quality of light in the greenhouse and growth chamber may have been of some influence on crawler movement.

Some plant species, such as collard, may be highly acceptable for feeding and offer readily available feeding sites for crawlers. A host such as cantaloupe may be highly acceptable for feeding and development, but the feeding sites might be less convenient to locate as compared with collard. The impediment or enhancement by trichomes on crawler travel activity were not specifically studied here. It is clear that factors other than trichomes were involved because there was varying crawling activity among the hosts (collard, cowpea, and pepper) that lacked trichomes. Temperature had no effect on net distance traveled by crawlers (Table 1). There was more variability in the data in the laboratory test compared with the greenhouse test. Nevertheless, during the greenhouse test, the lighting varied because of cloudy days during parts of the test, but the overall intensity of light was greater than in the growth chamber. The results in this study provide a better understanding of the behavior of whitefly crawlers on different vegetable hosts.

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